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R.L. DIMMICK, H. WOLOCHOW and M.A. CHATIGNY

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The purpose of this project was to conduct studies to determine whether bacteria, in or on small particles dispersed as aerosols, could maintain growth processes to the extent that more than one division step could occur. The question is important with respect to estimating the probability of contaminating the biological zone of Jupiter if space vehicles were to penetrate the planet. (Continued)

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An additional task was to determine whether metallic surfaces, simulating landing vehicle surfaces, would discharge attached microbes as a result of temperature changes.

The following findings, in chronological order, resulted from this study:

A. In the airborne state:

1. Bacteria ingested labelled glucose and produced labelled CO_2.

2. Bacteria ingested labelled thymidine and produced labelled DNA.

3. Phage was able to penetrate bacteria and, in one instance, additional phage appeared to have been formed.

4. Bacteria doubled in numbers when enclosed in droplets in the 1 - 3 μm diameter range.

5. Bacteria almost trebled in numbers when enclosed in droplets in the 3 - 6 μm range.

B. On a simulated Lander structure bacteria were found to be ejected from surfaces as a result of mechanical stress caused by temperature changes.

We conclude that, in an appropriate environment, bacteria could live indefinitely in the airborne state.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

FINAL REPORT

In fulfillment of Contract

#N00014-75-C-1133

R. L. Dimmick, H. Wolochow and M. A. Chatigny

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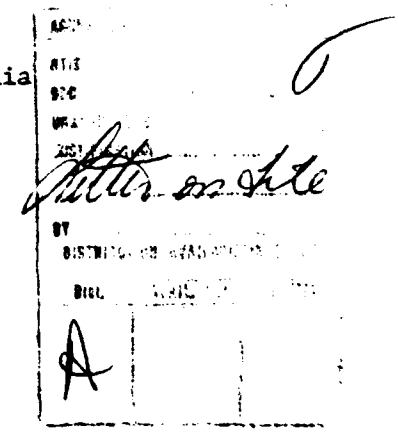
Naval Biosciences Laboratory

School of Public Health, University of California

Berkeley, California 94720



STUDIES ON MICROBIAL PROPAGATION IN THE AIRBORNE STATE

R.L. Dimmick, H. Wolochow and M.A. Chatigny

Naval Biosciences Laboratory
School of Public Health, University of California
Berkeley, California 94720

1. Objectives of Project

The purpose of this project was to investigate the possibility that microbes, airborne in or on small particles (1 μm to 100 μm diameter) could undergo cellular division under any circumstances. The study was initiated to gain additional information about the probability that the gaseous atmosphere of Jupiter could be contaminated by spacecraft from Earth. Projects associated with the major objective were to investigate:

(a) The possibility that, if cellular division occurred in air, it could be induced to continue for more than one generation,

(b) The possibility that airborne microbes could absorb nutrients from air,

(c) The possibility that anaerobic microbes could sustain functions similar to aerobes,

(d) The possibility that small microbial particles could be "shed" from metallic surfaces that were undergoing extreme temperature shifts,

(e) The possibility the the "lifetime" of a particle in the turbulent atmosphere of Jupiter might be sufficient to allow continued propagation.

2. Approach

Since the aerobiological literature of the past 30 years contained no reports of workers having observed evidence of division of cells in aerosol particles, the initial approach was to search for indirect evidence that might be immediately productive and that might provide information

to guide planning of direct, but more difficult experiments.

Proposed steps in an "If --, then --" sequence (with cells in the airborne state), were:

1. Find evidence of metabolism;
2. Find evidence of formation of new DNA;
3. Find evidence of competent genetic structure and function;
4. Find direct evidence of cell division;
5. Find indirect evidence for continued propagation (gravitational forces limits the time available).
6. Find direct evidence for continued propagation.

Major Accomplishments: Studies with Airborne Aerobic Bacteria

1. Active metabolic functions have been demonstrated (Appendices A, B).
2. Formation of new DNA has been demonstrated (Appendix C).
3. Marginally significant evidence for genetic integrity as shown by formation of phage has been found (Appendix D).
4. At least one generation of new cells has been demonstrated to form in small particles (average diameter 2 μm) (Appendix E).
5. Slightly more than two generations of new cells has been shown to form in large particles (4 to 6 μm diam.)(Appendix F).
6. Cup-shaped, metallic objects have been shown to release attached microbes in the form of small particles (Appendix G).

Minor Accomplishments, or those that have not shown affirmative data, include:

1. A method was developed to increase the coagulation rate of airborne particles (Appendix H).

2. Preliminary data from experiments in a large aerosol chamber indicated that particles decreased according to laws of stirred settling.

3. No evidence of metabolism of vegetative cells of anaerobes as aerosols in an anaerobic gas (N_2).

4. No evidence of division of anaerobic cells as aerosols in an anaerobic gas (N_2).

5. Contradictory evidence that anaerobic spores become heat sensitive as aerosols in anaerobic gas (N_2).

6. No evidence that airborne, aerobic cells can "feed" on other airborne particles or vapors.

7. A micro-aerophylic bacteria that grew at pH 10.6 in an ammonium atmosphere was isolated from soil. It failed to survive the 16th transfer and was not recovered.

During the project, 154 sets of aerosol runs (2 - 3 days per set) were conducted, most of which furnished negative or inconclusive data. In support of the runs, about 600 batches of cultures were produced and 4,850 samples were assayed.

CONCLUSIONS

1. Under special conditions of high humidity, growth temperature (30°C), suitable medium, and the use of cells in the latter stages of logarithmic growth, the bacterial species Serratia marcescens can sustain more than two cellular replications in the airborne state. Thus, the null hypothesis that no microbial species can replicate in the airborne state has been shown to be false.

2. Certain metallic structures undergoing dynamic thermal stress can discharge attached, viable, microbes in the form of small particles.

3. If the turbulent atmosphere of Jupiter is treated as a stirred settling chamber, that is, the boundaries are neither expanding nor contracting so the net vector velocities of air movement are zero, then stirred settling theory predicts the mean half-life of particles in the size range of 1 - 10 μm diameter would be between 5 to 10 years.

DISCUSSION

There are a number of factors associated with any attempts to estimate the probability that Jupiter would be contaminated by microbes unintentionally released from spacecraft entering the Jovian atmosphere. The factor of interest to this project was whether growth (in the sense of continued cellular division) is possible under any circumstance.

Prior to these studies, the best estimate of that probability was either nil or so close to nil that there was no practical difference. Our work has shown that this probability is actually 1, but the environment under which propagation can occur is not likely to be found on earth, and even less likely on Jupiter. It is important to note, however, that because of practical restrictions imposed by gravitational forces and chamber size, our studies were limited to 4-day periods at best. But growth usually ceased after 6 to 8 hours, indicating that essential nutrients had been consumed. In most instances, however, there was no additional death of airborne cells.

In view of the above, we believe that aerobic, airborne cells could be "fed", either continuously at a slow rate, or intermittently, by either nutrients in the vapor state, or by very small particles that would collide with the larger particles at a greater rate than if bacterial size particles were used. We might reasonably expect to find that the division time of airborne cells is longer than that of cells in vitro, so we might look for that process in cells 3 or 4 days (aerosol time) old. Limited studies of this nature are being continued.

Because it is possible for many aerobic cells to produce an ultimate waste product consisting of volatile products (CO_2 and water), whereas anaerobic cells, generally, do not easily rid themselves of waste materials, the possibility of demonstrating airborne growth of anaerobic microbes -- assuming there might be a species so oriented -- seems remote.

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APPENDIX G. Part I: RELEASE OF BACTERIAL SPORES FROM THE INNER WALLS OF A STAINLESS STEEL CUP SUBJECTED TO THERMAL STRESSES AND MECHANICAL SHOCK.
H. Wolochow, M. Chatigny and J. Hebert
In: 48th Technical Progress Report, Naval Biomedical Research Laboratory, School of Public Health, University of California, Berkeley, California. pp. 363-385

Part II: RELEASE OF BACTERIAL SPORES FROM INNER WALLS OF A STAINLESS STEEL CUP SUBJECTED TO THERMAL STRESS.
H. Wolochow, M.A. Chatigny and J. Hebert
In: 49th Technical Progress Report, Naval Biomedical Research Laboratory, School of Public Health, University of California, Berkeley, California. pp. 358-374.

APPENDIX H. A SIMPLE METHOD FOR ESTIMATION OF COAGULATION EFFICIENCY IN MIXED AEROSOLS
R.L. Dimmick, Alvin Boyd and H. Wolochow
Journal of Aerosol Science (1975) 6:375-377.

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